Skeletal Development: Vertebral Column and Limbs

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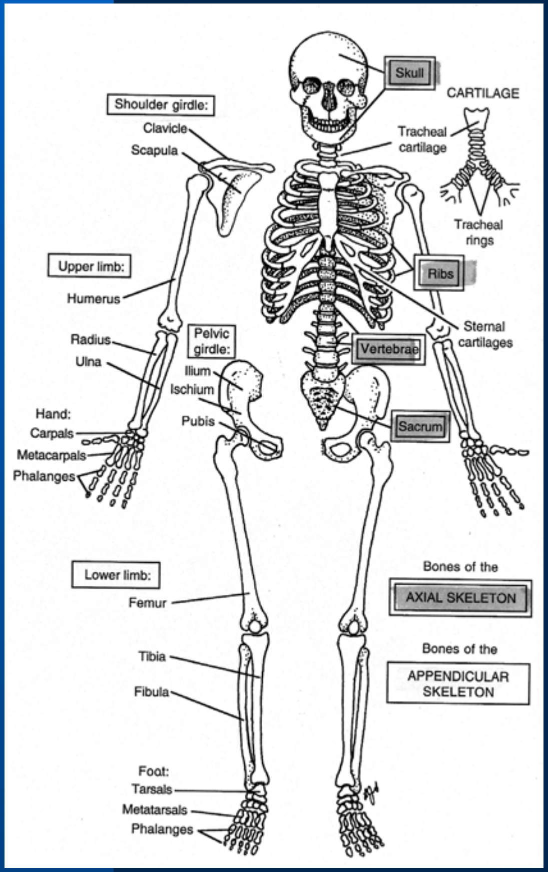
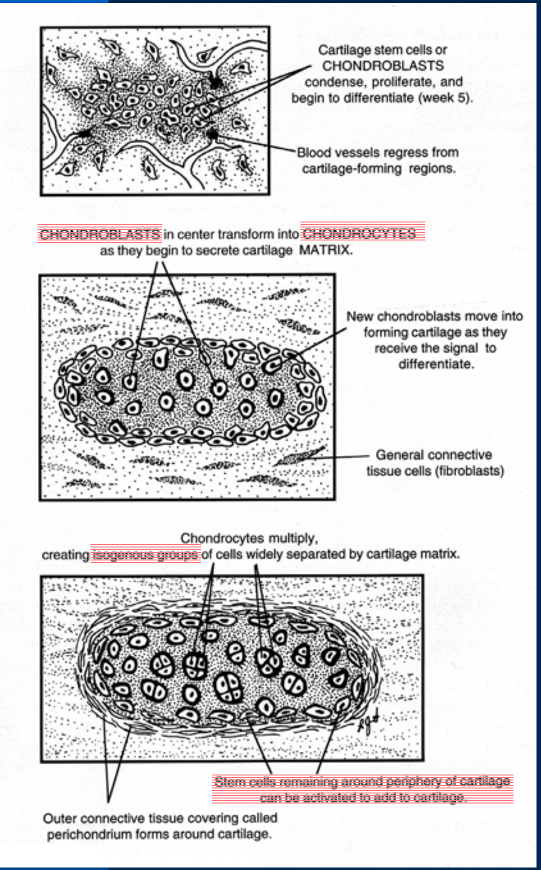
Axial and Appendicular Skeleton



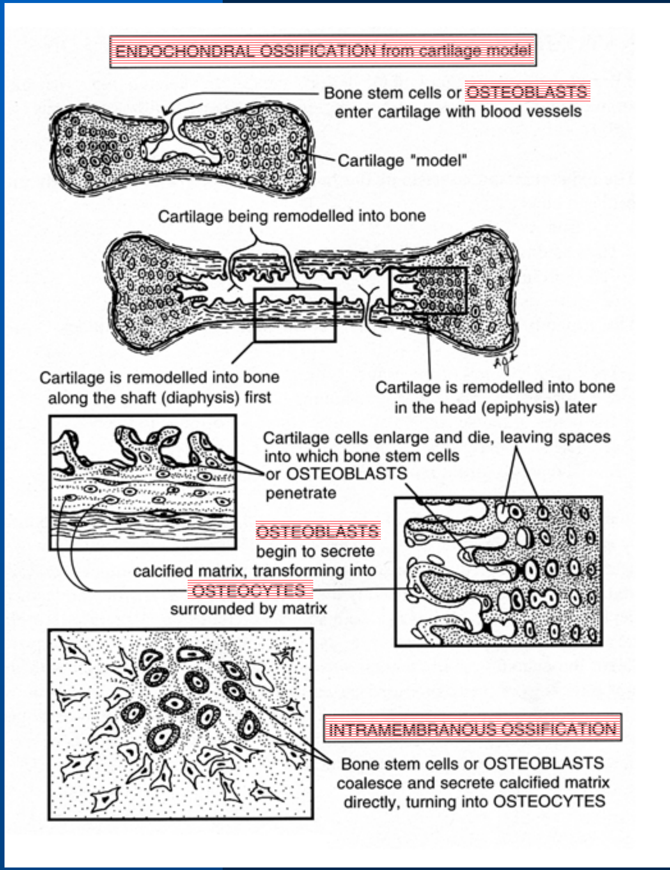
- 206 distinct bones in adult skeleton
- Axial skeleton
  - Vertebrae and ribs
  - Skull
- Appendicular skeleton
  - Appendicular girdles
    - Shoulder girdle
    - Pelvic girdle
  - Intrinsic limb bones

Cartilage Formation



- Beginning in <u>week 5</u>
  - *Chondro<u>blasts</u>* condense, proliferate, and begin to differentiate into *chondro<u>cytes</u>*
  - Deposit gelatinous matrix
  - Blood vessels regress (cartilage is avascular)
  - Chondrocytes divide and form isogenous groups ("nests")
- Cartilage growth by adding cells from external surface (perichondrium)
- Types of cartilage
  - *Hyaline cartilage* (e.g., synovial joints)
  - *Fibrocartilage* (e.g., intervertebral disks)
  - *Elastic cartilage* (e.g., auricle of ear)

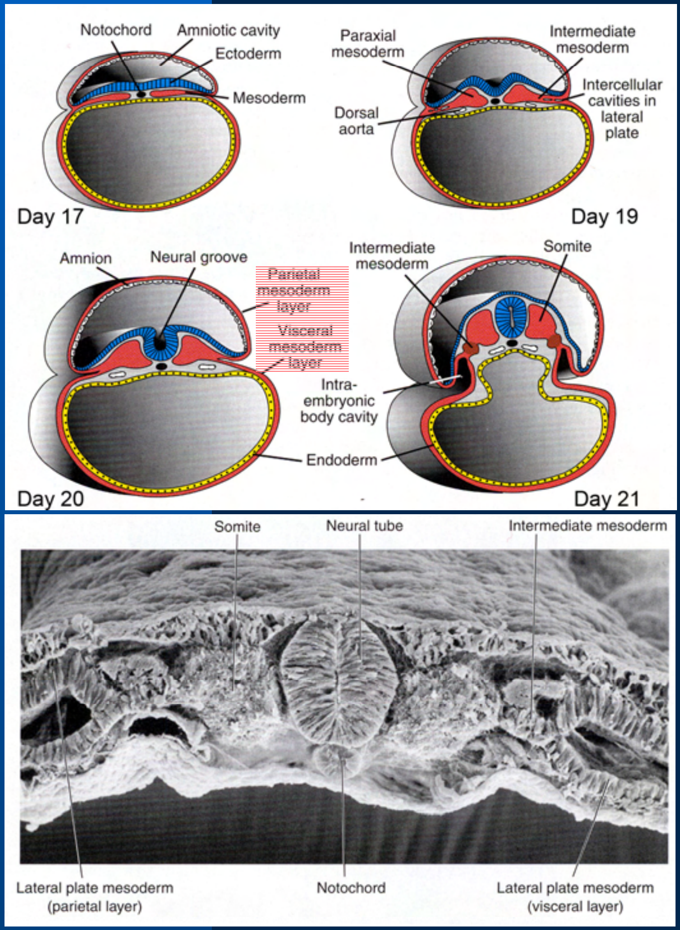
Bone Formation (Ossification)



Beginning in fetal month 3, bone is formed by two methods:

- Endochondral ossification
  - Bones formed from cartilagenous models
  - Cartilage cells die and leave spaces for osteoblasts and blood vessels
  - Ossification complete in early 20s
- Intramembranous ossification
  - For superficial flat bones in head; also for clavicle
  - Bone formed in intramembranous mesenchymal condensations of osteoprogenitor cells
  - Membranous sheets secrete bone matrix directly

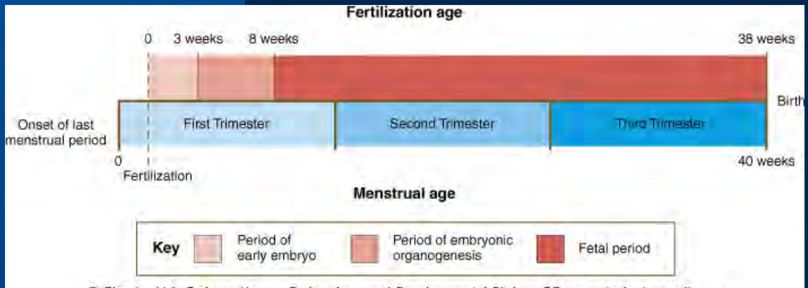
Derivatives of Mesodermal Germ Layer



Week 2: Mesodermal sheet condenses to form three parts

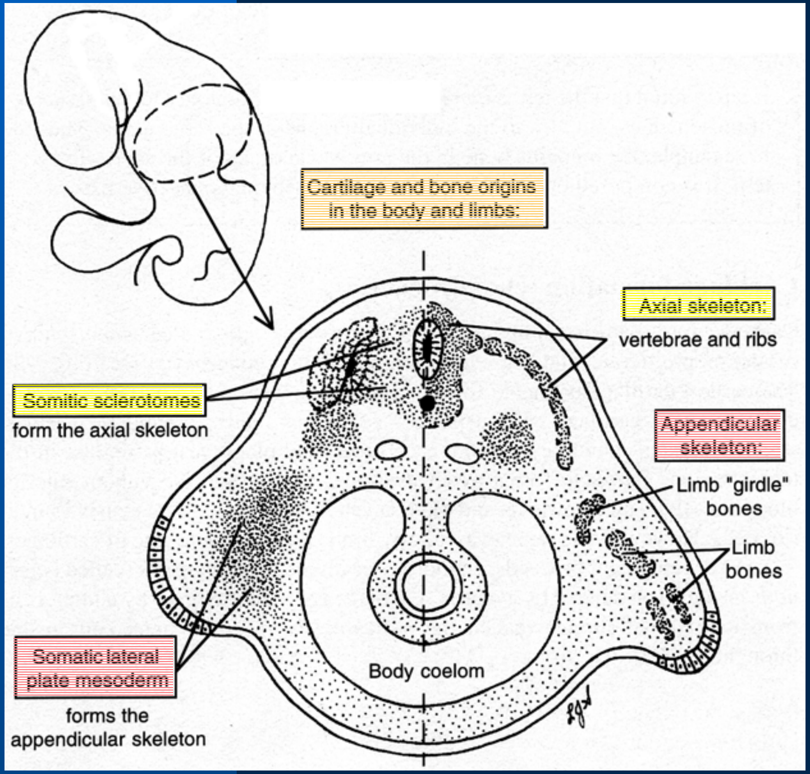
- Paraxial mesoderm
  - Future somites
- Intermediate mesoderm
  - Connects paraxial and lateral plate mesoderm
- Lateral plate mesoderm
  - Lines intraembryonic cavity
  - Parietal and visceral layers

Skeletal Development



- Week 4: Skeletal development begins in the embryonic period
- Cartilage and bone derived from mesoderm
  - Different subdivisions of mesoderm give rise to cartilage/bone of different regions of body
- Many bones develop from pre-existing cartilage models

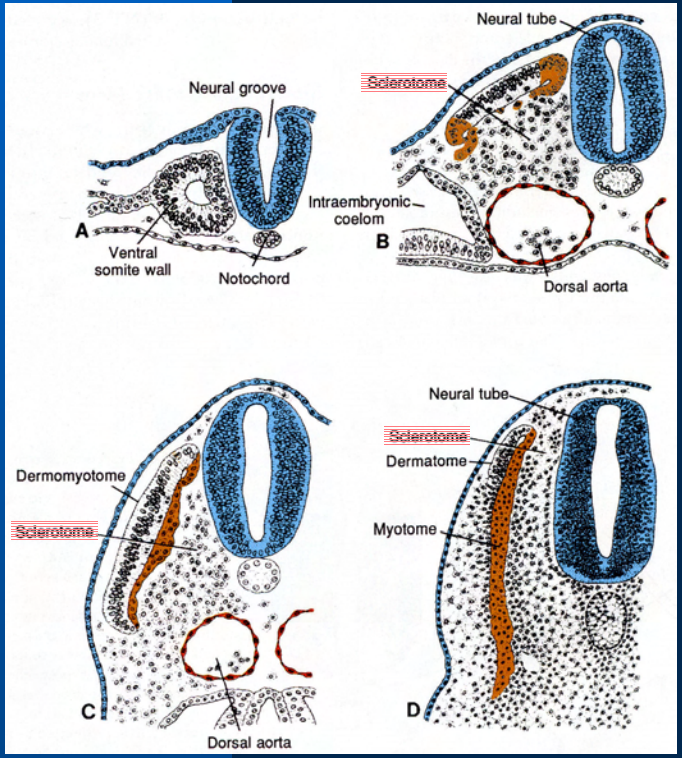
Mesoderm Origins of Cartilage and Bone



- Scleretome (somitic)
  - Forms axial skeleton in body (some in head)
    - NOTE: Somitic mesoderm is in somites
- Lateral plate mesoderm (somatic)
  - Forms appendicular skeleton
  - Forms body wall (*soma)*
    - NOTE: Somatic mesoderm is in the body (soma)

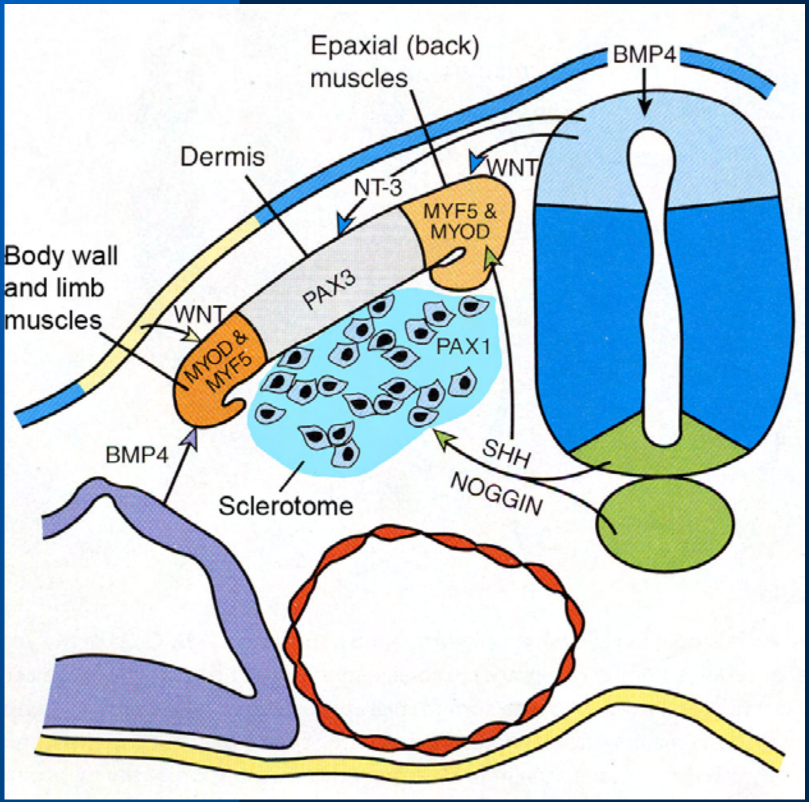
Development of Axial Skeleton

Somite Development and Axial Skeleton



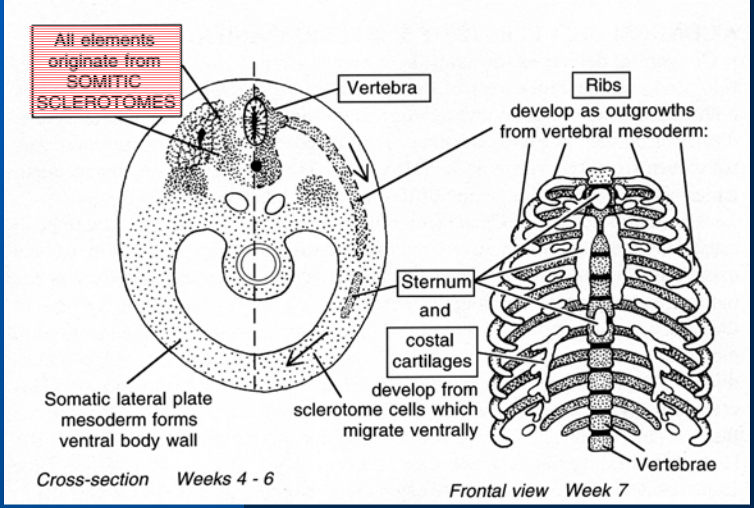
- Mesoderm around small cavity
- Week 4: Ventromedial cells migrate toward notochord to form sclerotome (mesenchyme)
  - Some cells form tendons
  - Surround spinal cord and notochord to form vertebral column

Gene Expression in Somite Differentiation



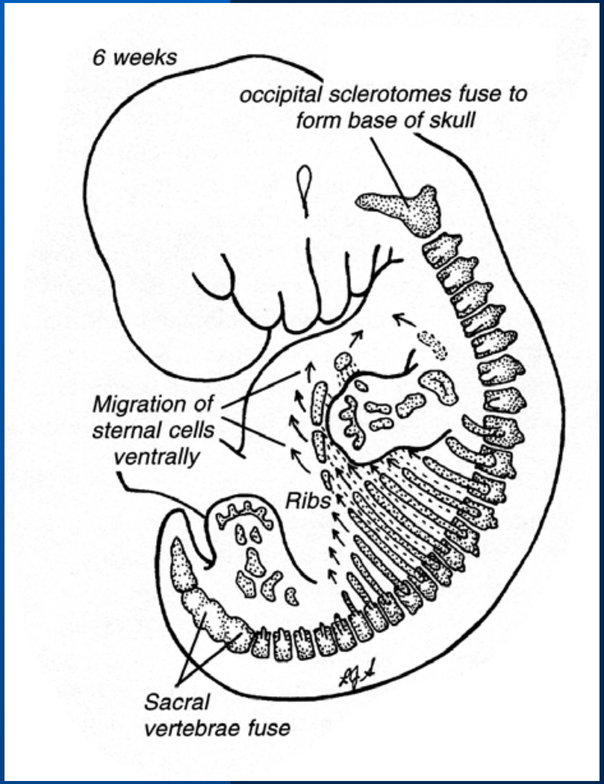
- Proteins *noggin* and *sonic hedgehog (SHH)* secreted by notochord and neural tube floor plate
  - Induce sclerotome formation
- Once induced, scleretome cells express PAX1
  - PAX1 initiates a cascade of cartilage- and bone-forming genes for vertebral column development

Origin of Vertebrae, Ribs, and Sternum



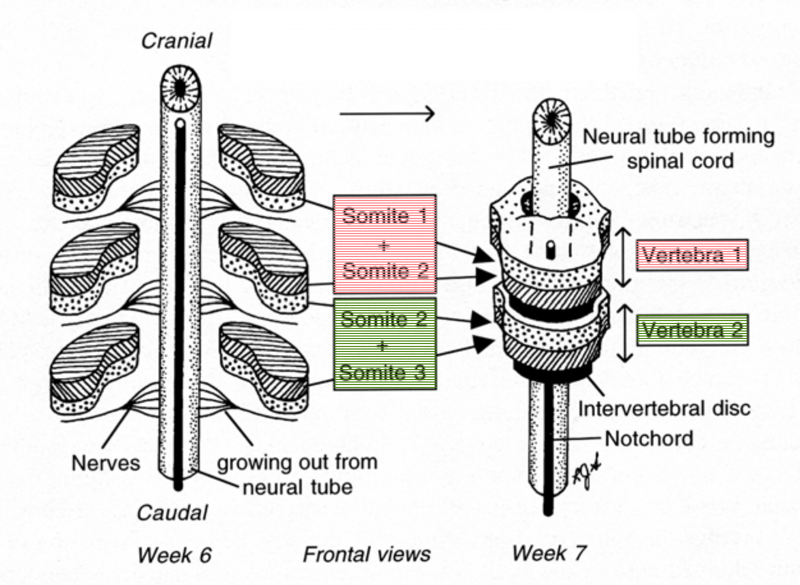
- Vertebrae
  - Formed by endochondral ossification of sclerotome regions
  - Sclerotome from each side of body contributes to each vertebra and intervertebral disk
- Ribs
  - Outgrowths of lateral (costal) processes of thoracic vertebrae
- Sternum
  - Ribs 1-7 unite ventrally to form sternum
- Costal cartilages
  - Ribs joined to sternum by costal cartilages

Axial skeleton formation



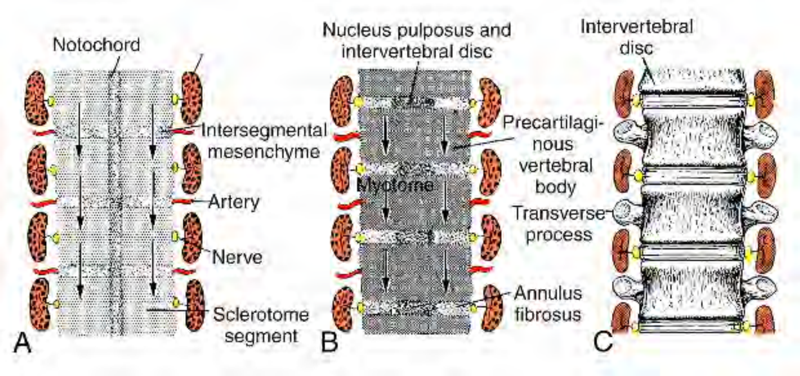
- 42-44 somite pairs
- Develop cranial → caudal sequence
  - *Occipital*: 4
    - 1 degenerates
  - *Cervical*: 8
    - First cervical and remaining occipital somites fuse to form base of skull
  - *Thoracic*: 12
  - *Lumbar*: 5
  - *Sacral*: 5
    - Fuse to form sacrum
  - *Coccygeal*: 8-10
    - Last 5-7 degenerate
    - Remainder fuse to form coccyx

Vertebral Column Formation



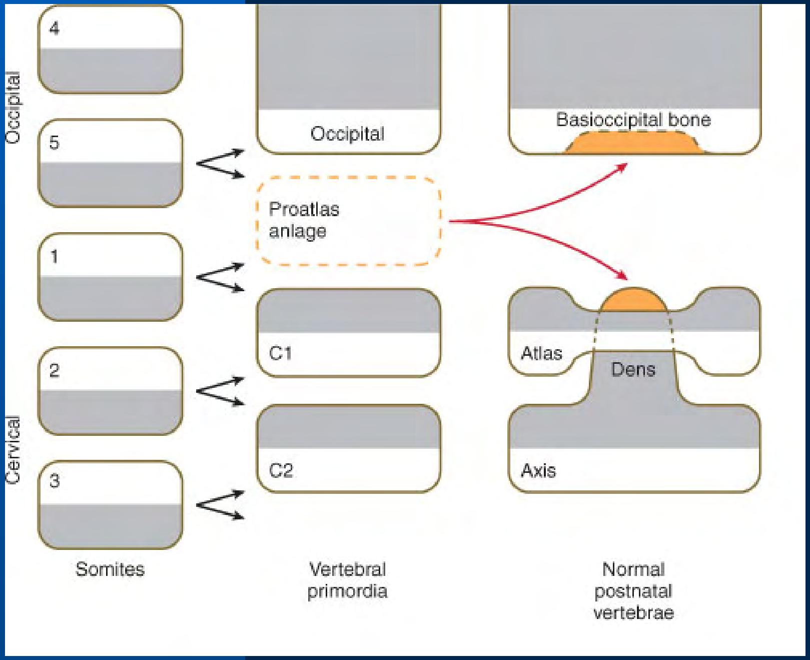
- Vertebrae form by fusion of sclerotome cells from two somite levels
- Week 4: Sclerotome cells surround developing neural tube and notochord
- Ventral part of each vertebra
  - Forms centrum around notochord (future body)
- Dorsal part of each vertebra
  - Forms costal processes (future transverse processes) and vertebral (neural) arch
    - In thoracic region, costal processes articulate with ribs

Formation of Intervertebral Disks



- Intervertebral disks formed between vertebrae by condensation of other sclerotome cells
  - Notochord persists and enlarges to form nucleus pulposus
  - Surrounded later by annulus fibrosus)
  - Notochord regresses completely in region of vertebral body
- Resegmentation of sclerotome into vertebrae
  - Causes myotomes to span intervertebral disks & gives them capacity to move vertebral column
  - Intersegmental arteries (originally between sclerotomes) pass over middle of vertebral body
  - Spinal nerves near intervertebral disks; pass through intervertebral foramen

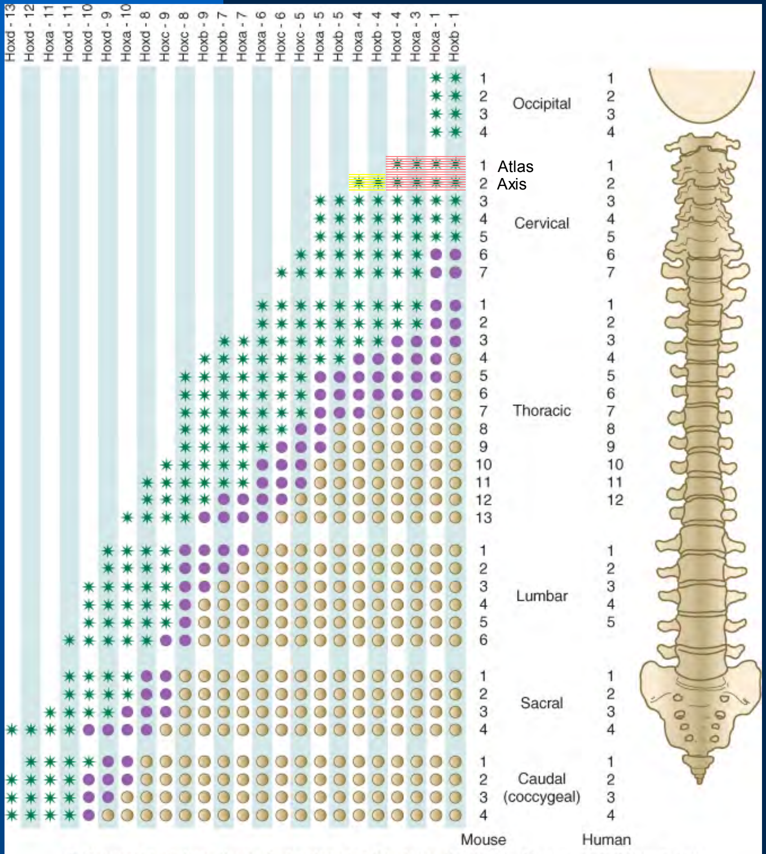
Formation of Atlas (C1) and Axis (C2)



Formation of atlas and axis in mice

- Cells from “proatlas” contribute to formation of basioccipital bone and dens
- Normal atlas forms anterior arch (transient in other vertebrae) instead of centrum (body)
- Cells that would normally form centrum of atlas (C1) fuse with axis (C2) to form dens

Molecular Control of Vertebra Development



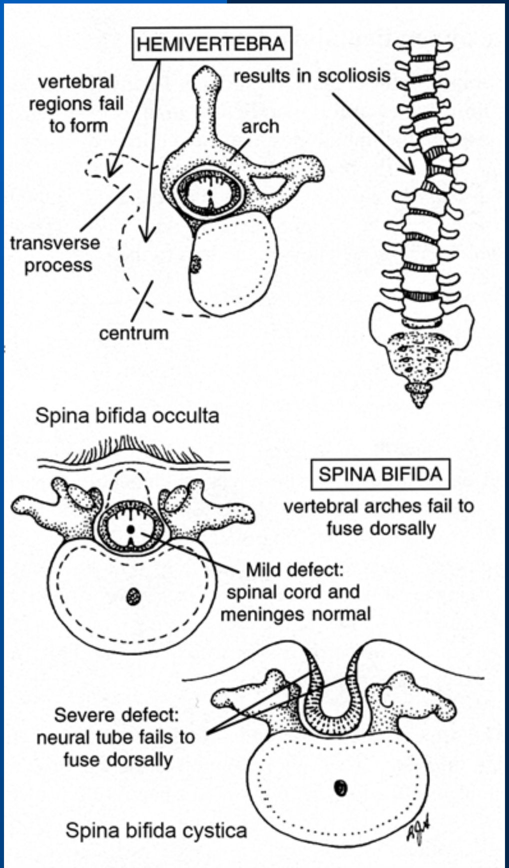
- SHH stimulates sclerotome cells to express *PAX1*
  - Guide ventromedial sclerotome cells to form centrum
- Induction of roof plate of neural tube results in *PAX9*, *MSX1*, and *MSX2* expression
  - Guide lateral sclerotome cells to form dorsal (vertebral) arch
- Individual vertebrae specified by combinations of *Hox* genes

  Example
  - Atlas (C1): *Hoxa-1, Hoxa-3, Hoxb-1, Hoxd-4*
  - Axis (C2): *Hoxa-1, Hoxa-3, Hoxb-1, Hoxd-4* + *Hoxa-4 & Hoxb-4*

Klippel-Feil Syndrome (*Brevicollis*)

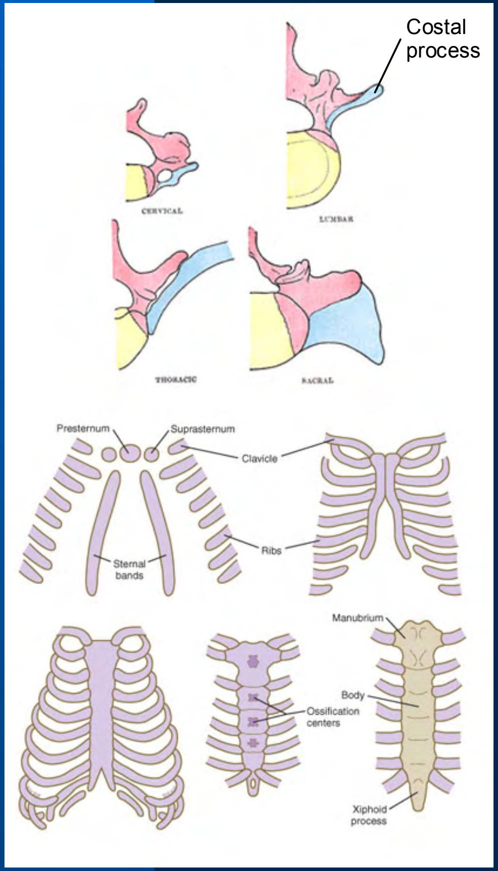
- Rare condition characterized by patients with:
  - Short neck (*brevicollis*)
  - Decreased cervical motion
  - Low hairline
  - ✓ (<50% with C-spine defects have all three signs)
- Congenital failure of segmentation of cervical vertebrae
  - 3-8 weeks: Failure of normal cervical somite segmentation
  - Result: multiple fused cervical segments (esp. C2 & C3)
- Familial Klippel-Feil gene locus on chromosome 8
- Associated anomalies
  - Scoliosis (60%)
  - Hearing loss (36%)
  - GU abnormalities (34%)

Axial Skeleton: Vertebral Defects



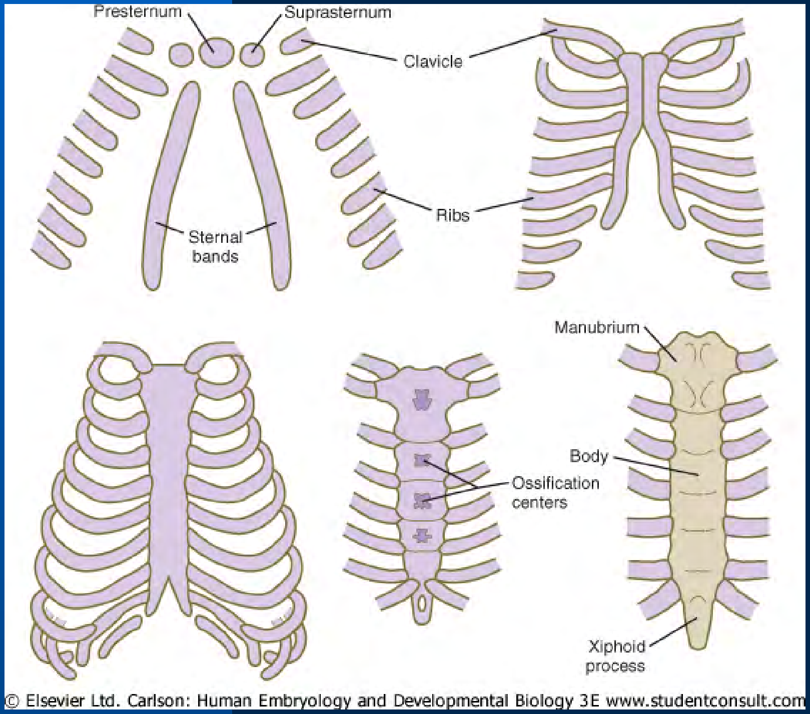
- Congenital defects often result from faulty induction (e.g., *Hox* gene misexpression)
  - Excess retinoid acid (RA)
    - Multivitamins or anti-acne medications
  - Cranio-caudal RA gradient activates *Hox* genes
    - $\uparrow$RA $\rightarrow$ Hox expression shifts
    - Type of vertebra to inappropriately more caudal type
  - Metabolic disturbances (e.g., high glucose or excess insulin in maternal diabetes
- Spina bifida (*split spine*)
  - Incomplete development of vertebral arch
    - 1° cause: failure of normal vertebral induction
    - 2° cause: failure of neural tube to close dorsally
  - Herniation of spinal cord and/or meninges
- Scoliosis (G. *skolios* = bent)
  - Defective formation of one side of vertebral column
  - Hemivertebra: only half of vertebra forms

Rib Development



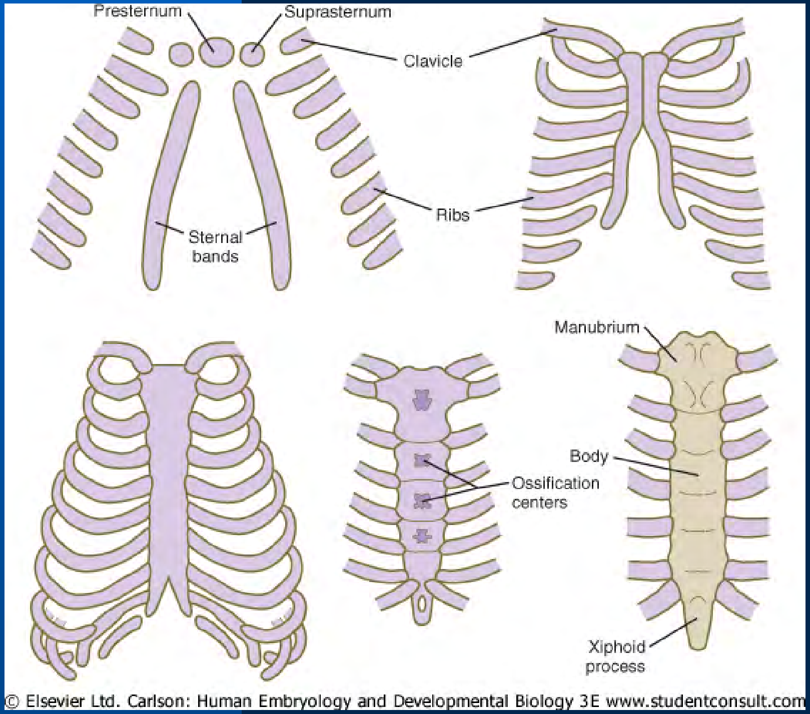
- 12 pairs of ribs develop during month 2
  - Outgrowths of costal (lateral) processes of thoracic vertebrae
- Sclerotome cells migrate within somatic lateral plate mesoderm around body curvature toward ventral surface
- Accessory ribs
  - Upper lumbar or lower cervical
  - <1% incidence
  - Probably result from misexpression of *Hox* genes

Sternum Development



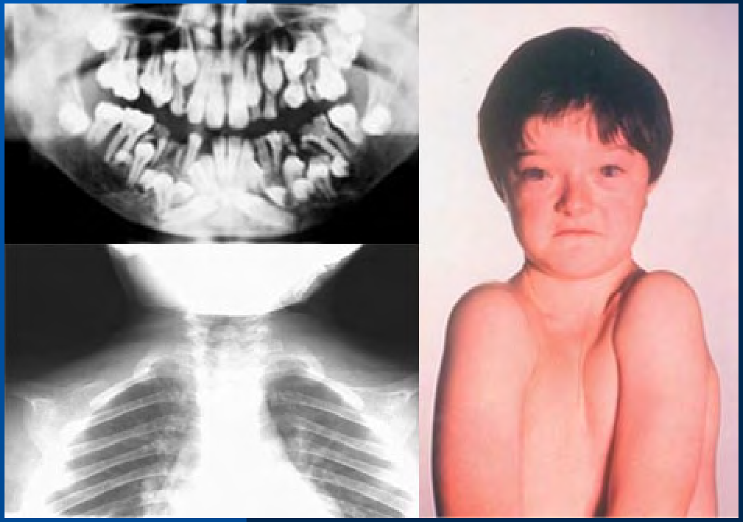
- First 7 ribs unite to form sternum (also formed from migrating sclerotome cells)
  - Ribs joined to sternum by costal cartilage
- Arise from pair of cartilagenous bands around ventral midline
  - Fuse to form cartilagenous model of manubrium, sternbrae (4), and xiphoid process
- Divides secondarily into elements
  - Division similar to formation of synovial joints seen elsewhere
- Split xiphoid process a common malformation
  - Mutations in *Hox* genes

Clavicle Development



- Weeks 5-6: Ossification begins
- 21-yo: Ossificaton complete
  - One of the first bones in body to ossify
- Forms by intramembranous ossification

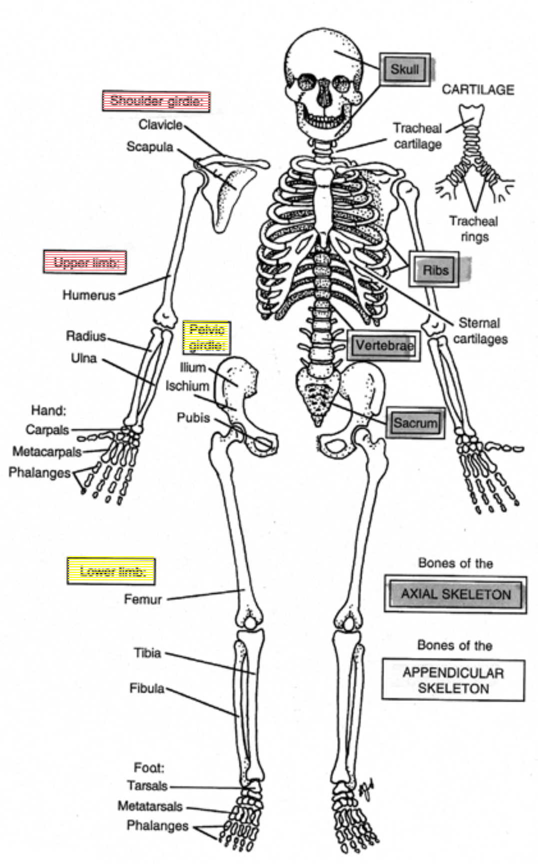
Cleidocranial dysplasia



- Abnormality affecting skeletal development of head, dentition, and clavicle
- Craniofacial malformations
  - Nose flat, wide, and lacking a bridge
  - Presence of supernumerary and unerupted teeth
  - Primary dentention may be retained into adulthood
- Aplasia or hypoplasia of clavicles
- Prevalence: 1/1,000,000

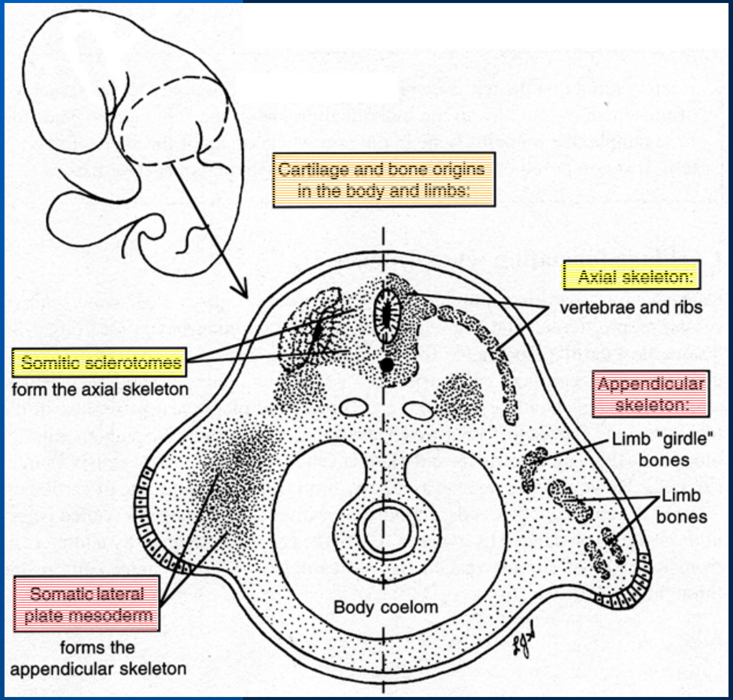
Development of Appendicular Skeleton

Appendicular Skeleton



- Appendicular skeleton
  - Appendicular girdles (shoulder & pelvic)
  - Intrinsic limb bones
- Appendicular skeleton formed by endochondral ossification
  - Exception: clavicle forms by intramembranous ossification

Origins of Cartilage and Bone in Limbs

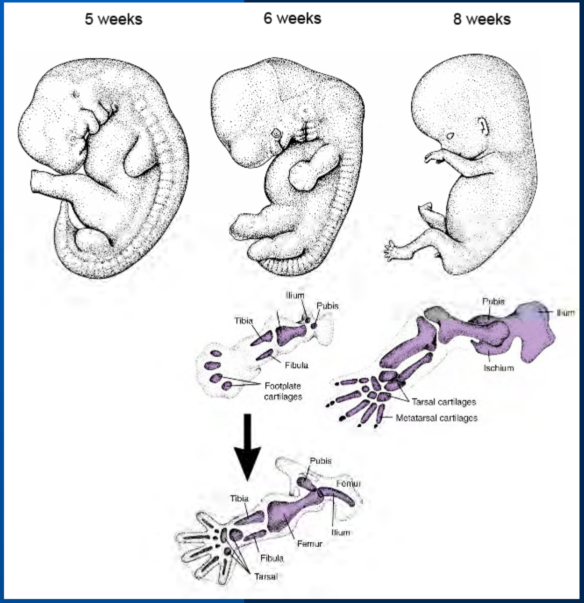


Somitic mesoderm is in somites

Somatic mesoderm is in the body (*soma*)

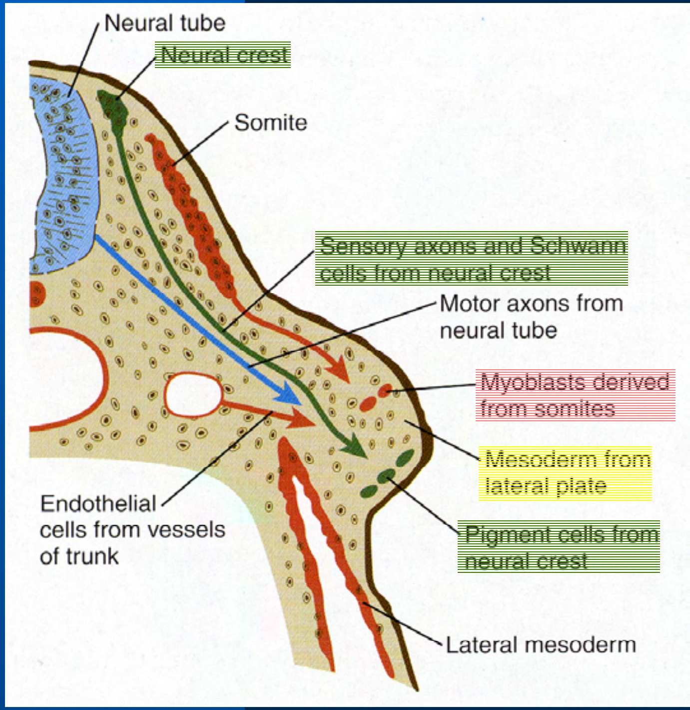
- Mesoderm condenses into three regions
  - Paraxial
  - Intermediate
  - Lateral plate
- Somatic lateral plate mesoderm
  - Forms all cartilages and bones of appendicular skeleton
  - Forms body wall (*soma)*
- Somitic scleretome
  - Forms all cartilages and bones of axial skeleton in the body (some in head)

Limb Development



- End of <u>week 4</u>
  - Limb buds visible
- Mesenchyme core
  - Derived from somatic layer of lateral plate mesoderm
- Surrounded by ectoderm
  - Hindlimb buds less well developed that those of forelimb (approx. 1-2 days later)
- <u>Week 5</u>: Condensation of cartilage mesenchyme
- <u>Week 6</u>: Cartilage models begin to form
- <u>Week 8</u>: Cartilage models completed and ossification begins
- <u>Weeks 13-16</u>: Active ossification of fetal skeleton

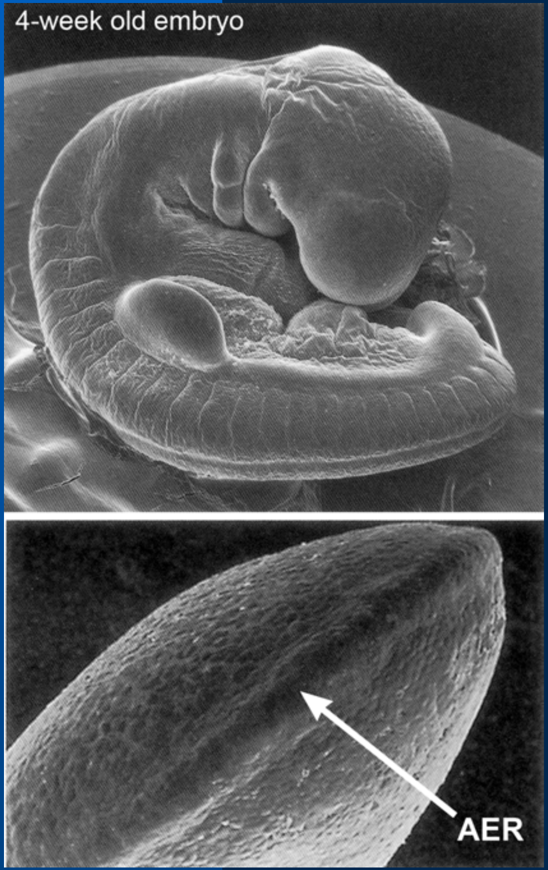
Limb Bud Mesoderm



Limb bud initially consists of:

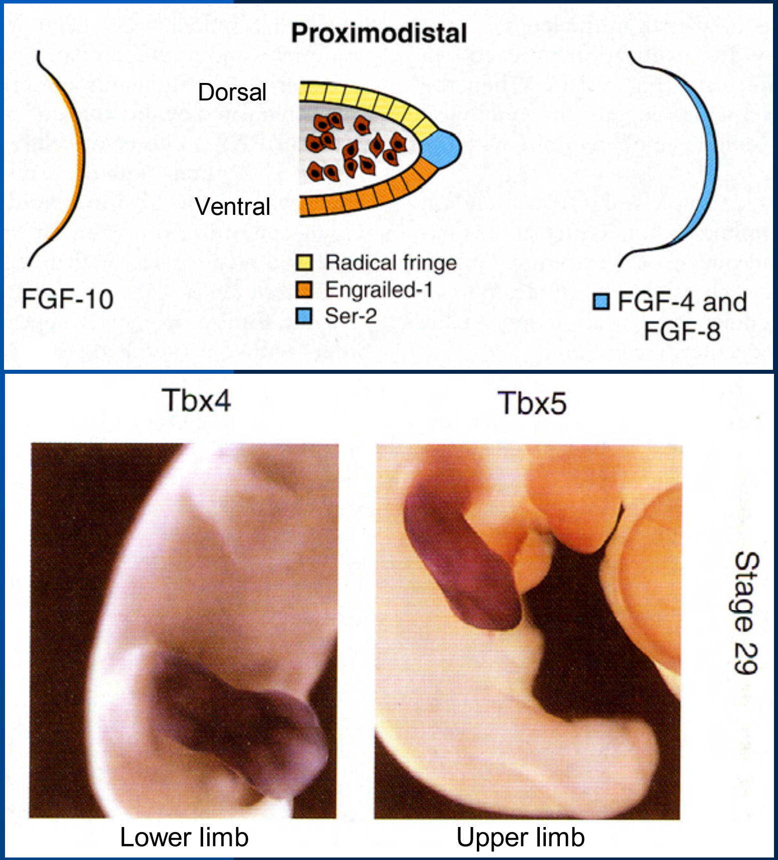
- Mesenchyme from lateral plate mesoderm
  - Forms skeleton, connective tissue, and some blood vessels
- Mesenchymal from somites
  - Form muscle
- Neural crest cells
  - Form Schwann cells and melanocytes (pigment cells)

Apical Ectodermal Ridge (AER)



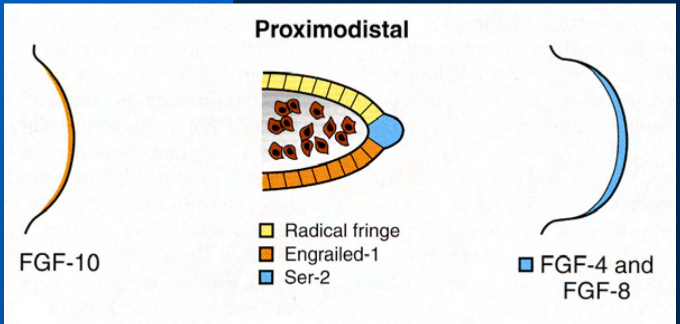
- Apical ectodermal ridge
  - Thickened distal border of limb bud
  - Exerts inductive influence on underlying mesenchyme
    - Prevents its differentiation and causes it to proliferate rapidly
  - Dorsal ectoderm
    - Expresses radical fringe (signaling molecule)
  - Ventral ectoderm
    - Expresses engrailed (En-1) (transcription factor)

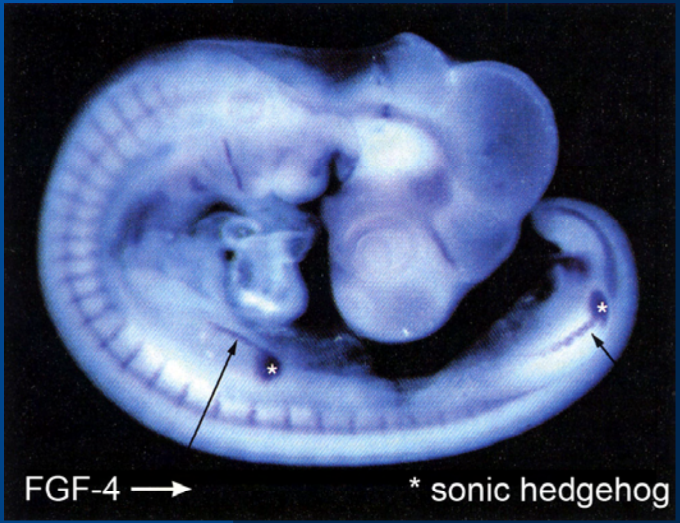
AER Formation



- Limb outgrowth initiated by factors secreted by lateral plate mesoderm in limb-forming regions
- Upper Limb
  - FGF10 + TBX5
- Lower Limb
  - FGF10 + TBX4

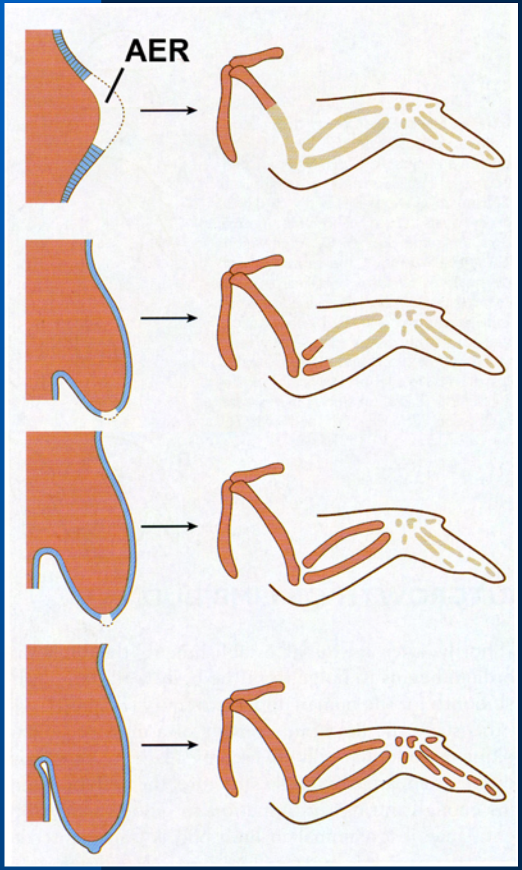
AER and Limb Elongation





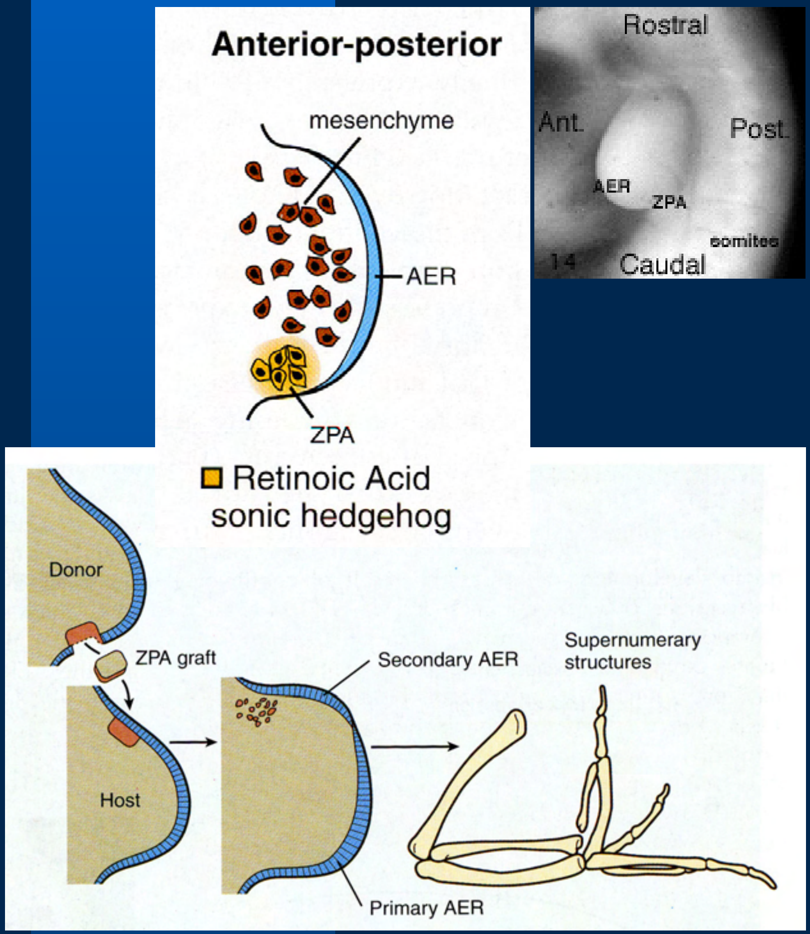
- BMPs (in <u>ventral</u> ectoderm) induce AER formation
  - Signaling through homeobox gene *MSX2*
- AER restricted to distal tip of limb by radical fringe (<u>dorsal</u> ectoderm)
- In turn, radical fringe induces *SER2*
  - Expressed in AER cells
- Engrailed-1 (<u>ventral</u> ectoderm) repress expression of radical fringe to maintain border
- After AER established, its cells express FGF4 and FGF8
  - Maintain "progess zone" of rapidly proliferating mesenchymal cells adjacent to AER
- As limb elongates, proximal mesenchymal cells become farther from influence of AER
  - Decrease their rate of division
  - Begin differentiation

Arrested Limb Development and AER



- Removal of AER at successively later stages of development
  - Results in arrest of limb development (distal truncation of limb)

Zone of Polarizing Activity (ZPA)



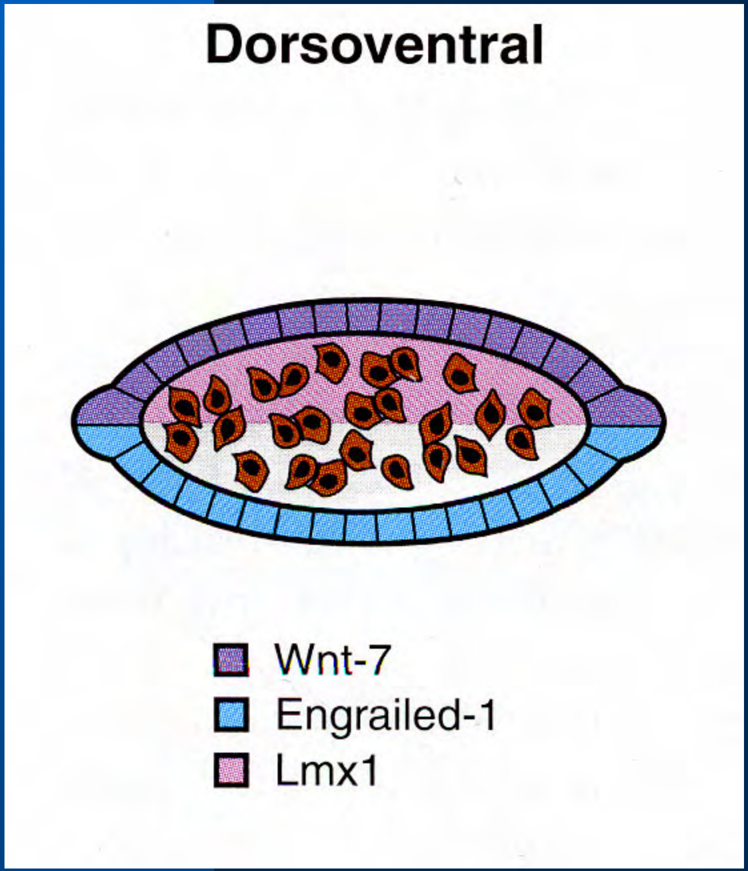
- ZPA is a cluster of cells at posterior border of limb (near flank)
  - Location determined by transcription factors (*Hoxb-8, Gli-1, Gli-3*)
  - Signaling center along A/P axis of limb
- ZPA cells produce retinoic acid (RA) which initiates expression of sonic hedgehog
  - Absence of SHH → regression of AER
- ZPA determines proper location of digits (e.g., thumb on radial/anterior side)
- As limb grows, ZPA moves distal to remain in proximity to AER

Misexpression of RA or SHH results in mirror image duplication

Graft mesoderm (≤50 cells) from P → A margin

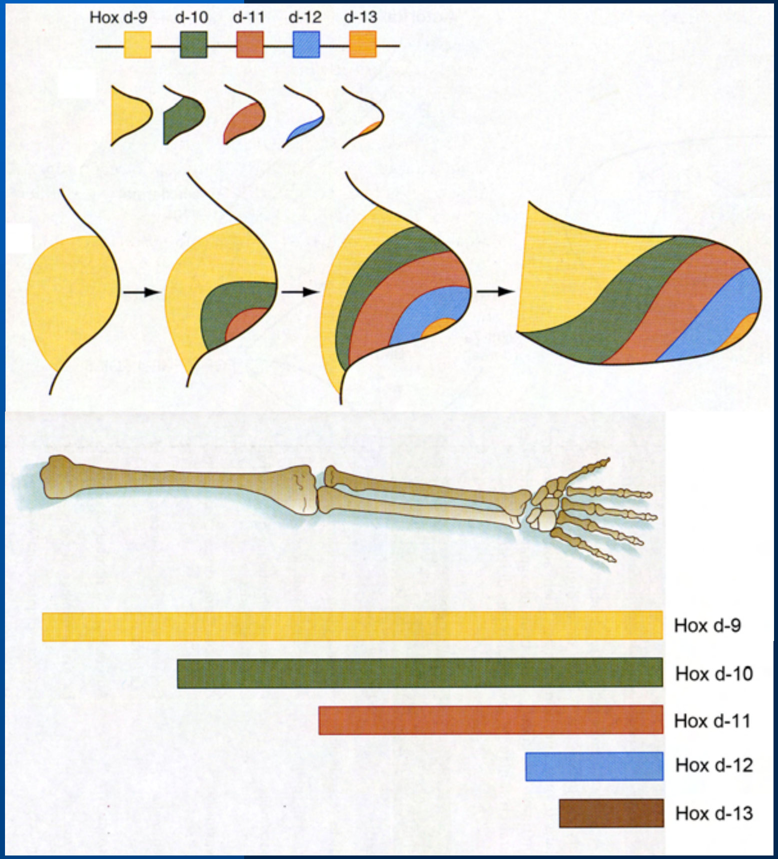
Formation of extra limb

Patterning along Dorsoventral Axis



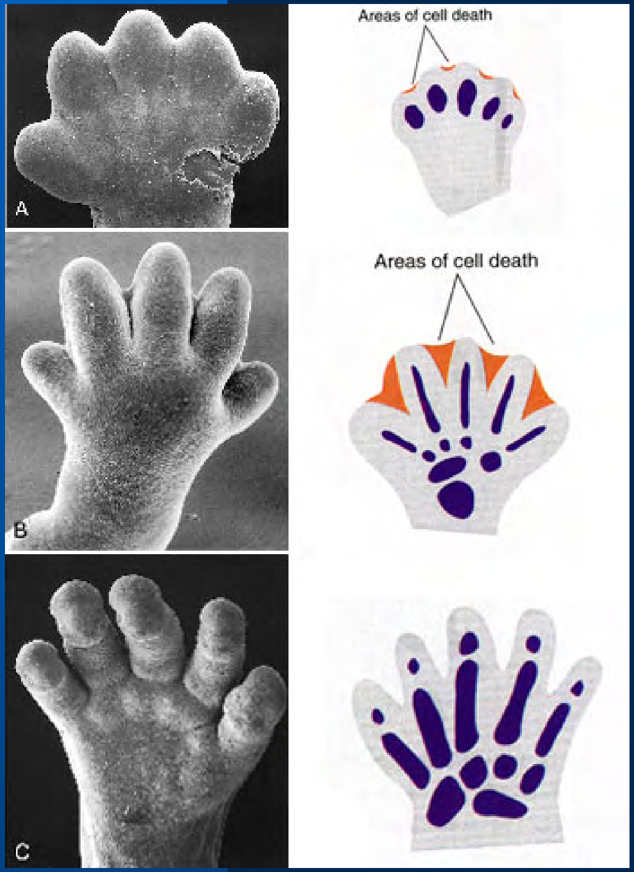
- Regulated by BMPs in ventral ectoderm
  - Induce expression of *EN1*
  - *EN1* represses *WNT7a* expression (restricting it to dorsal ectoderm)
  - *WNT7a* is a secreted factor that induces *LMX1* in dorsal mesenchyme
- *LMX1* specifies cells to be dorsal
- WNT7a also maintains SHH expression in ZPA (indirectly affects A/P patterning)

Hoxd Expression Patterns in Limb



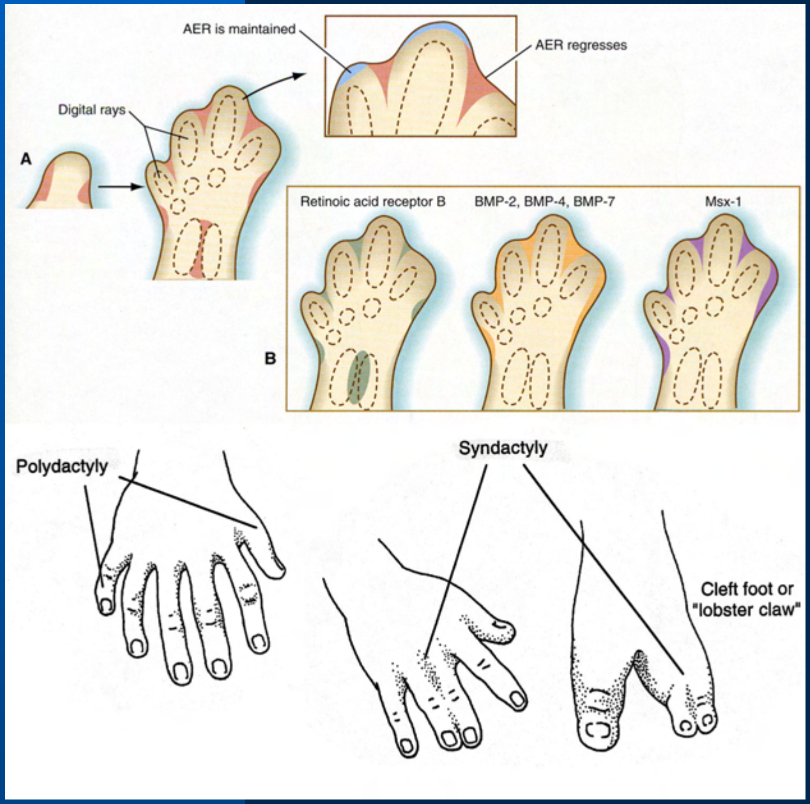
- *Hoxd* genes pattern limb along proximodistal axis
  - Pattern seen in embryonic and adult limb
- Specific *HOX* expression, results from combinatorial expression of SHH, FGFs, and WNT7a
  - Differences in combinations may account for differences in forelimb and hindlimb structures
- Mutations in *Hoxd-13*
  - Cause reduction defects of digits (short phalanges)

Cell Death and Development of Digits



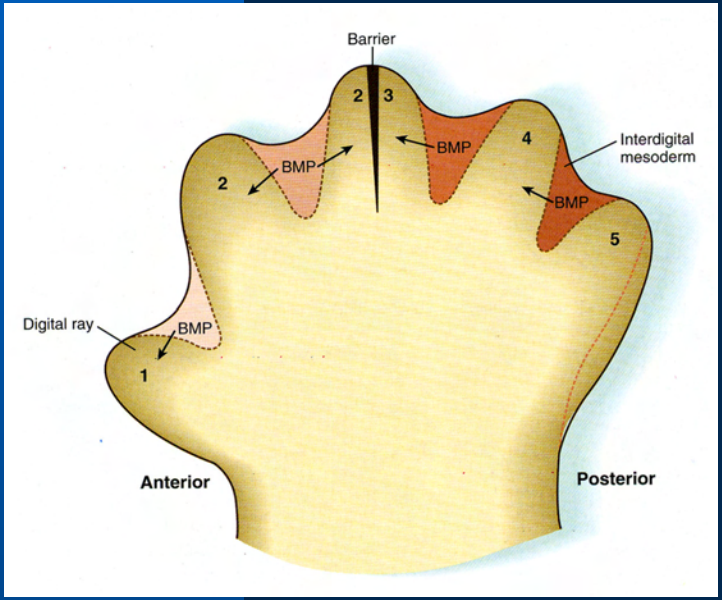
- 48 days
  - Genetically programmed cell death (apoptosis) in apical ectodermal ridge creates separate ridge for each digit
- 51 days
  - Cell death in interdigital spaces separates digits
- 56 days
  - Digit separation complete

Interdigital Cell Death



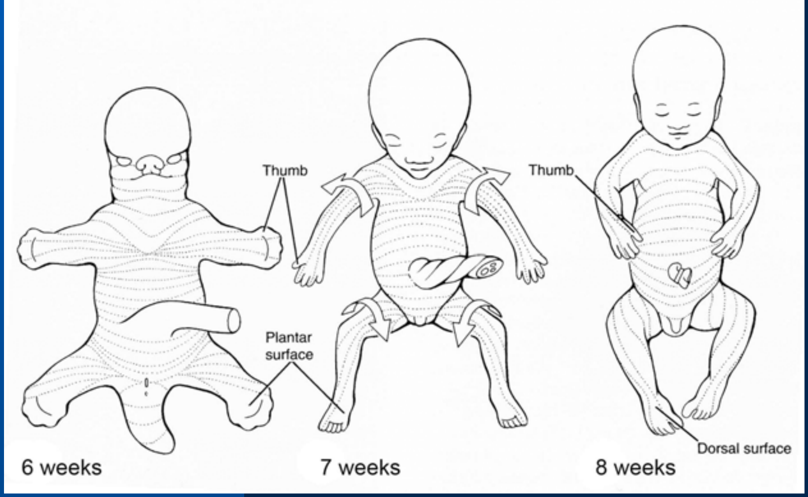
- Apoptosis plays a role in formation of:
  - Axillary region
  - Radius/ulna & tibia/fibula
  - Interdigital spaces
- Hand and digit formation
  - Balance between AER regression and maintenance
  - BMPs and *MSX* transcription factors in interdigital spaces
  - If cell death does not occur, syndactyly (abnormal digit formation) occurs

BMP and Digit Specification



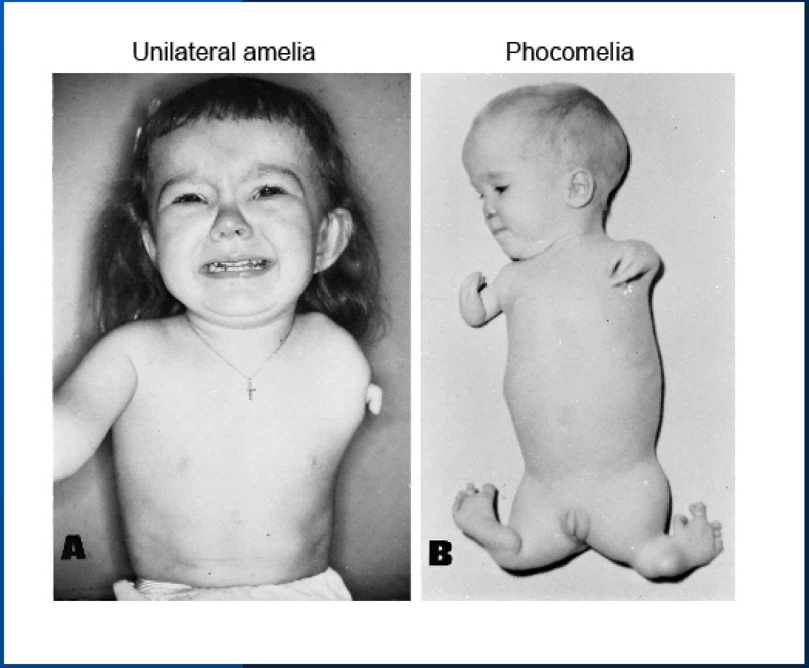
- BMP concentration determines digit identity
- Highest concentration
  - Specifies digit 4 (ring finger)
- Lowest concentration
  - Digit 1 (thumb)
- Specification of digit 5: *not known*
- Experiment:
  - Separation of digit 3 by a barrier
    - Posterior 1/2 → digit 3
    - Anterior 1/2 → digit 2 (supernumerary)

Limb Rotation



- Limb rotation occurs along the proximodistal axis
- Upper limb undergoes a rotation in an opposite direction to that of the lower limb
  - Upper limb rotates laterally
  - Lower limb rotates medially
- Result: homologous bones and muscles (including their actions) between the upper and lower limbs are 180° apart.
  - Flexors and extensors are, therefore, positioned opposite sides of the limb when comparing the lower limb from the upper limb.

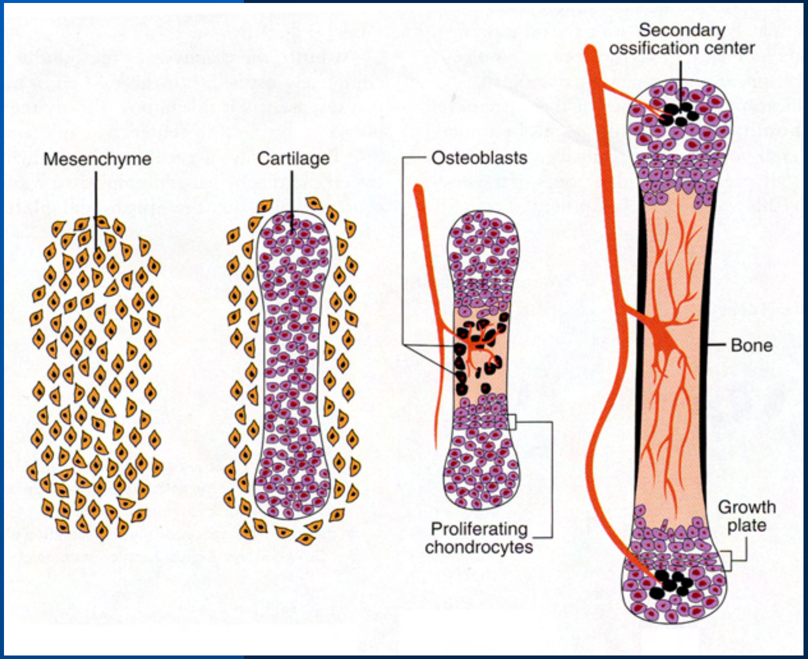
Amelia and Meromelia



✓Latin, *melos* = a limb
✓Greek, *meros* = part

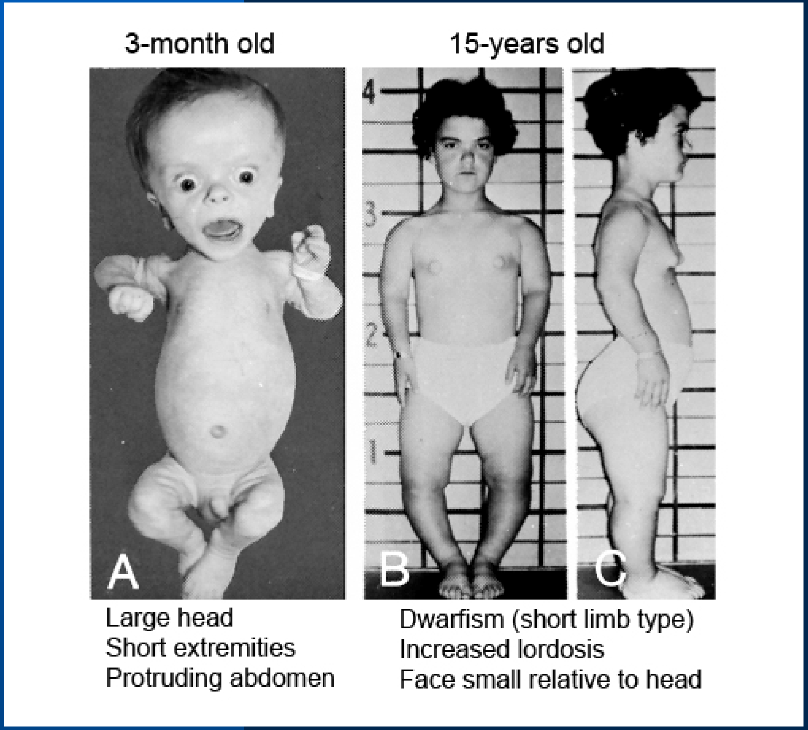
- Amelia
  - Complete absence of limbs
- Meromelia
  - Partial absence of limb
  - Phocomelia
    - Proximal structures (I.e., long bones) small or absent
    - Hands or feet attached to trunk by irregularly shaped bones

Endochondral Bone Formation



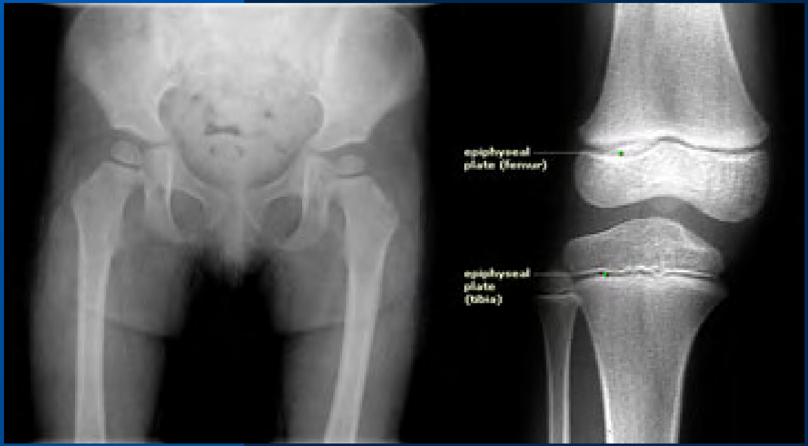
- Endochondral ossification begins at end of embryonic period
  - Week 12: Primary ossification centers present in all long bones
- Ossification proceeds from shaft (diaphysis) toward ends of cartilagenous model
  - At birth: diaphysis usually completely ossified but ends (epiphyses) still cartilagenous
- Shortly thereafter secondary ossification centers arise in the epiphyses

Achondroplasia (“Dwarfism”)



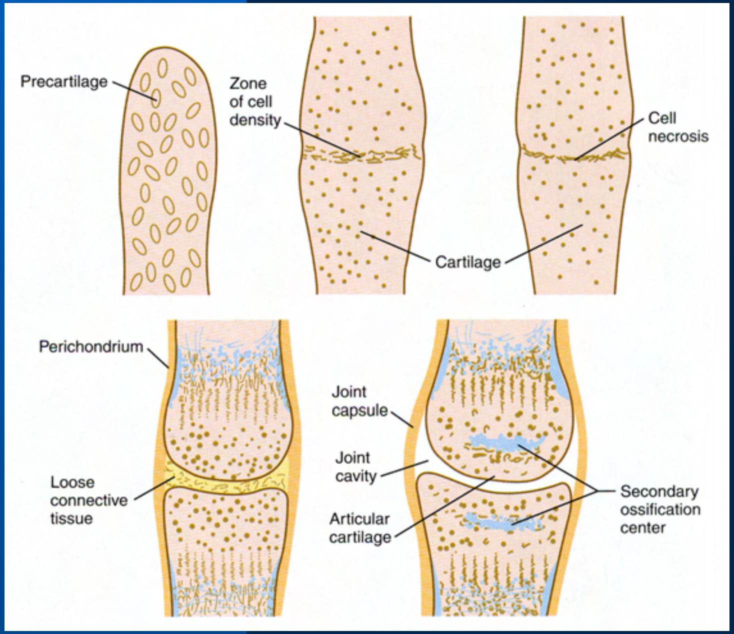
- Abnormal ossification of cartilage
- Predominantly affects long bones
- Epiphyseal growth retarded and ceases early
  - Dwarfism at birth
- Syn. achondroplastic dwarfism

Epiphyseal Plates



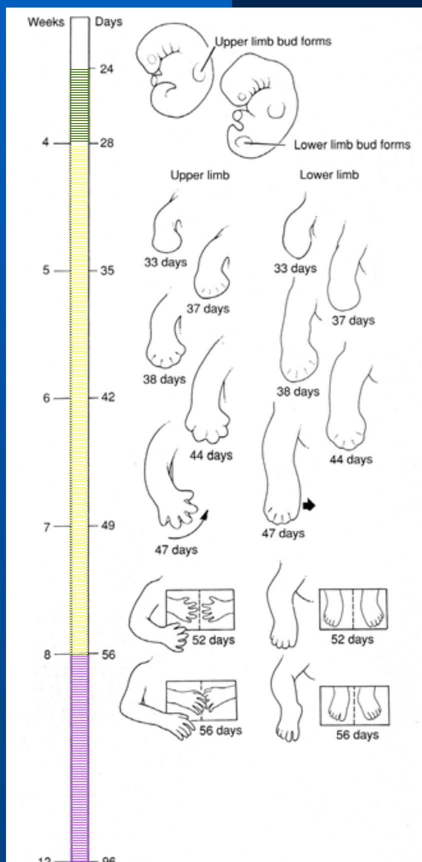
- Temporary cartilage (*epiphyseal* or *growth*) plate remains between diaphysis and each epiphysis
  - Epiphyseal plate at both ends of long bones
  - Smaller bones (e.g., phalanges) have only one
  - Irregular bones (e.g., vertebrae) have >1 primary ossification centers, and several secondary ossification centers

Joint Formation



- Joints formed in cartilagenous condensations when chondrogenesis is arrested
- Joint “interzone” induced
  - Cells proliferate and increase in density
- Joint cavity formed by cell death
- Surrounding cells form joint capsule
- WNT14 appears to be inductive signal

Limb Development - Overview



- 24 days
  - Upper limb buds
- 28 days
  - Lower limb buds
- 33 days
  - Hand plates visible
- Weeks 4-8
  - Limb morphogenesis
- Week 6
  - Cartilagenous models
- Week 7
  - Hand digital rays
- Week 8
  - Foot digital rays
- Weeks 8-12
  - Ossification begins